PDGF receptor-mediated signal transduction: from nm to cm

Jason Haugh

http://www.che.ncsu.edu/haughlab/

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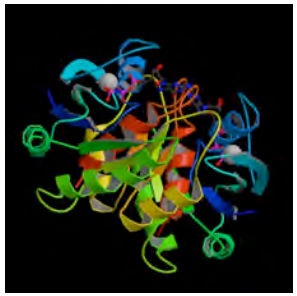
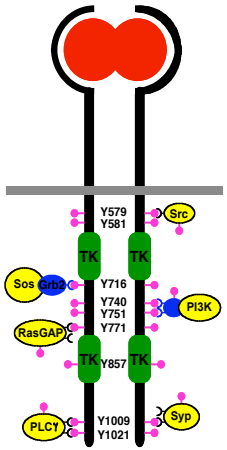
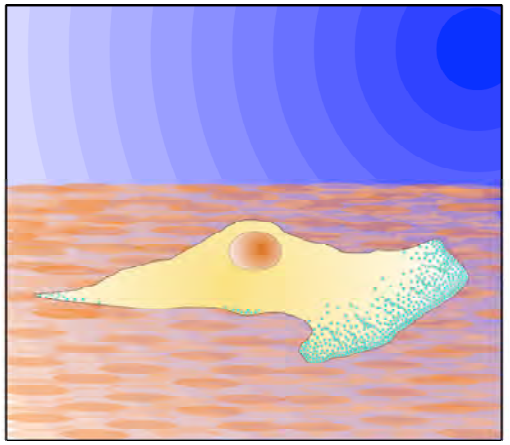
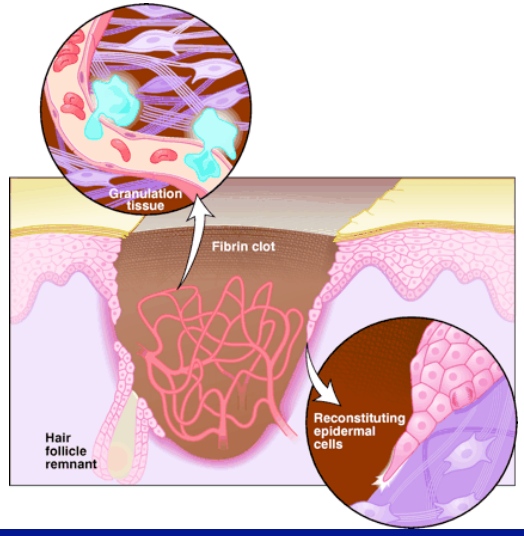
Integration of biochemical and biophysical processes across multiple scales of complexity



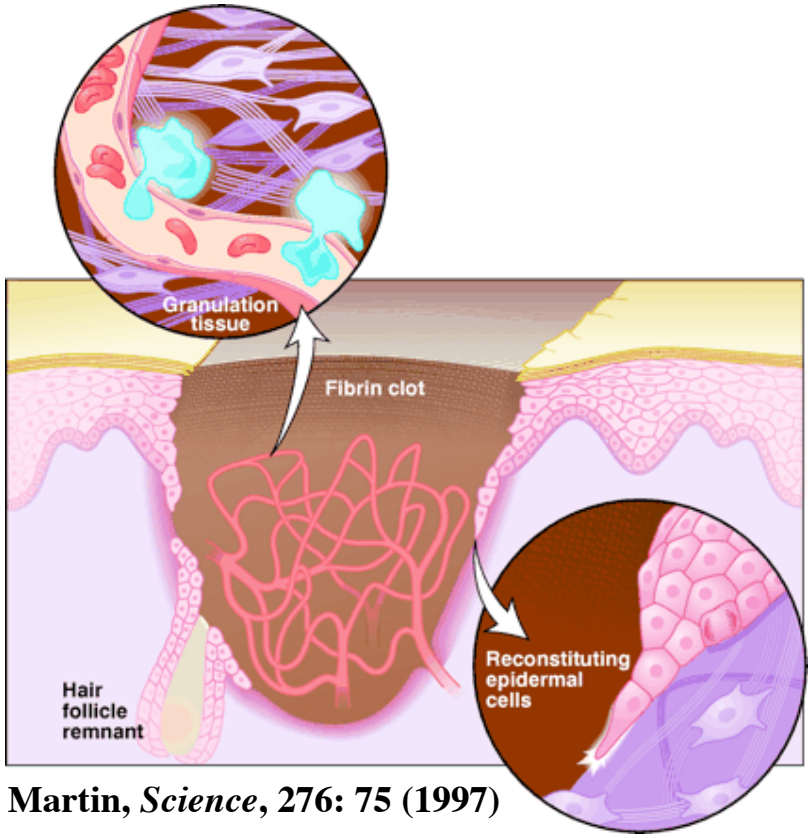


1. Molecular structure/function
2. Protein activation/ localization states
3. Biochemical signaling pathways
4. Cell behavior and function
5. Integrated, tissue-level response

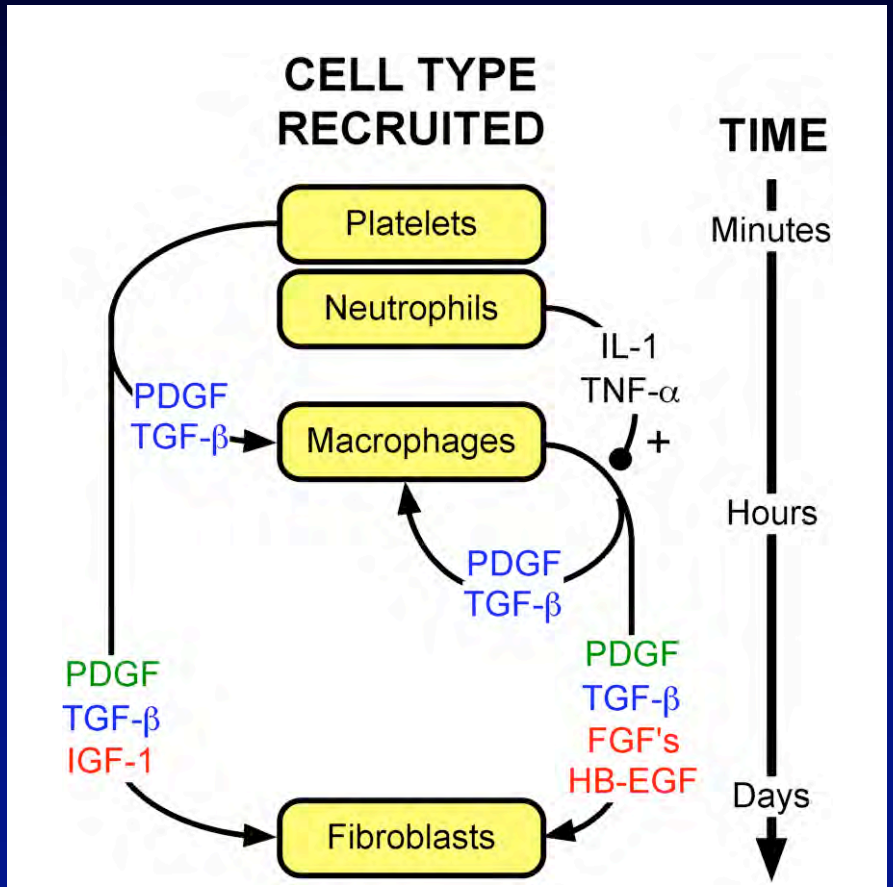




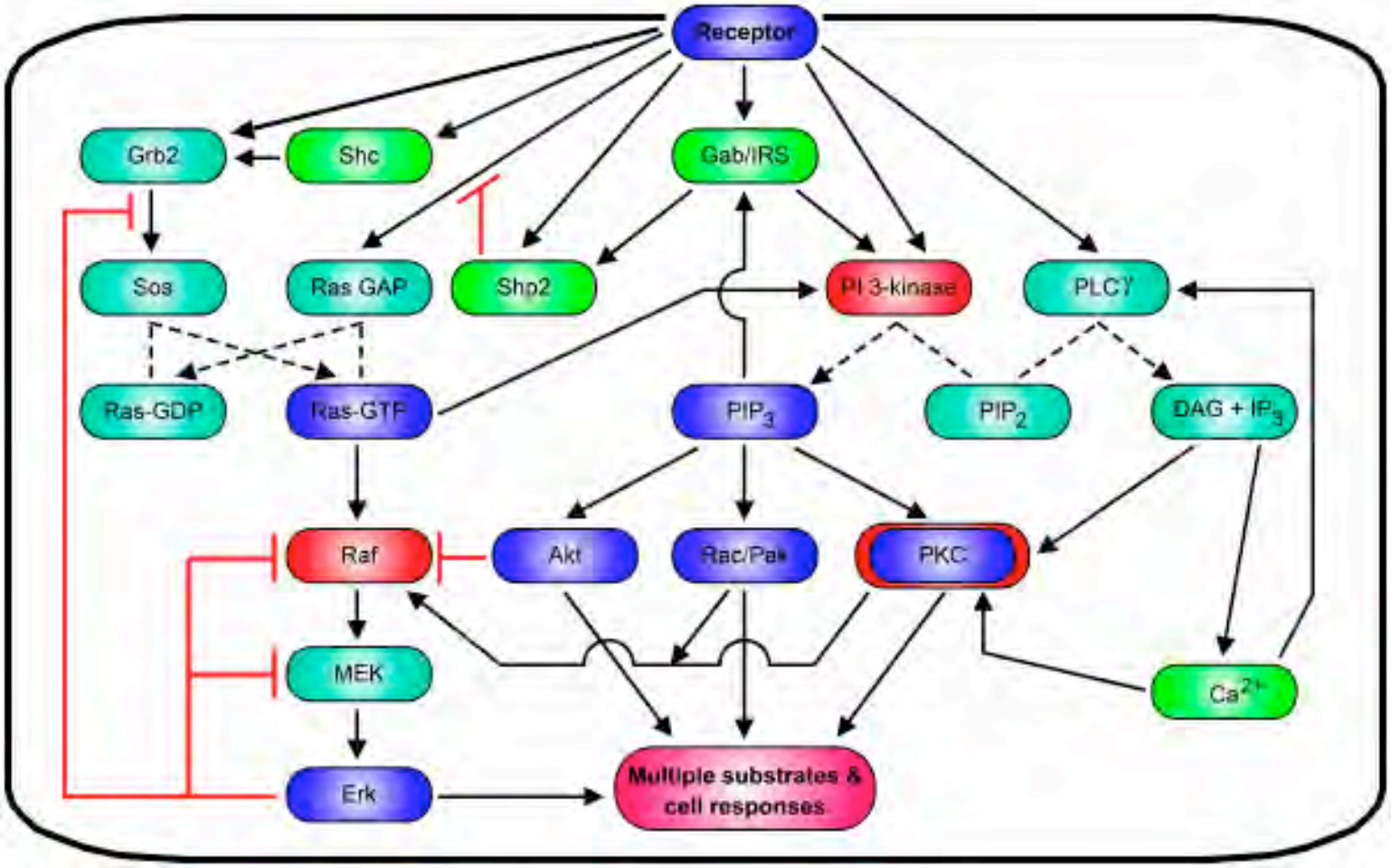
The wound healing cascade



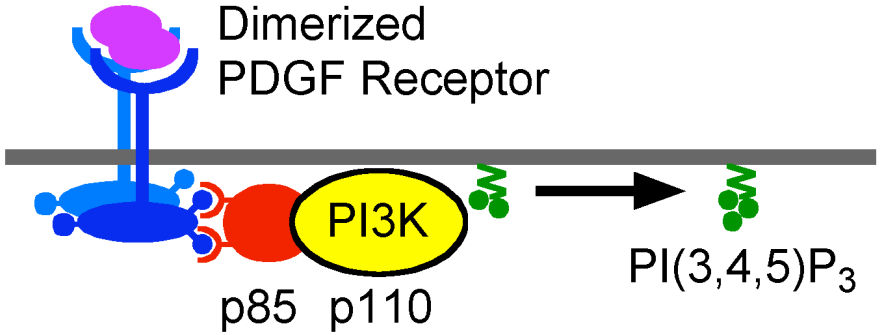
Martin, *Science*, 276: 75 (1997)



Manipulation and kinetic analysis of crosstalk in the PDGF receptor signaling network (C.C. Wang)

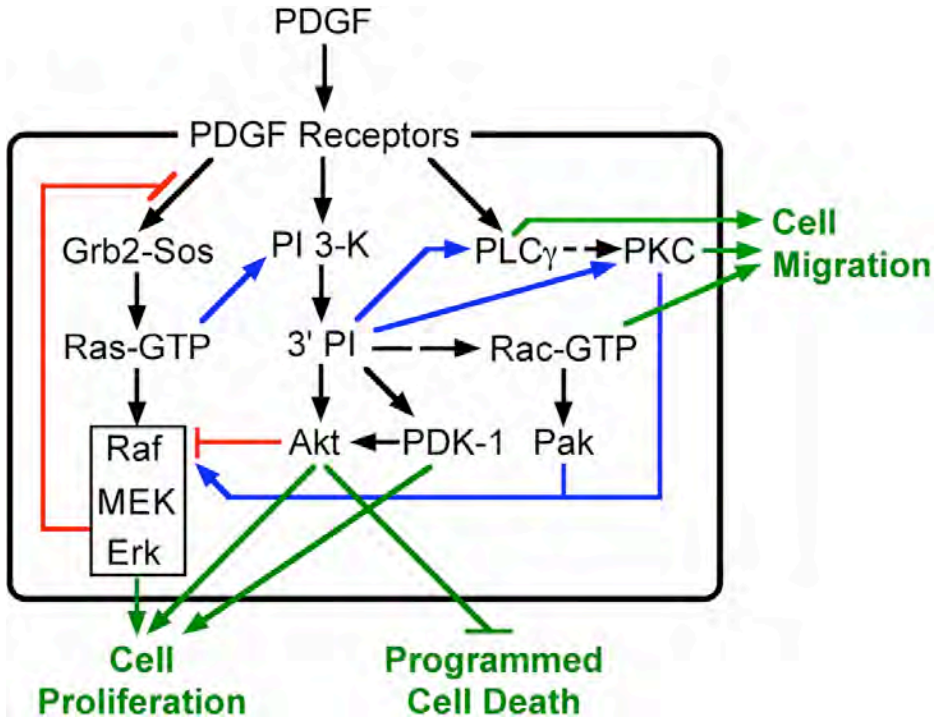


PI 3-kinase signal transduction in fibroblasts



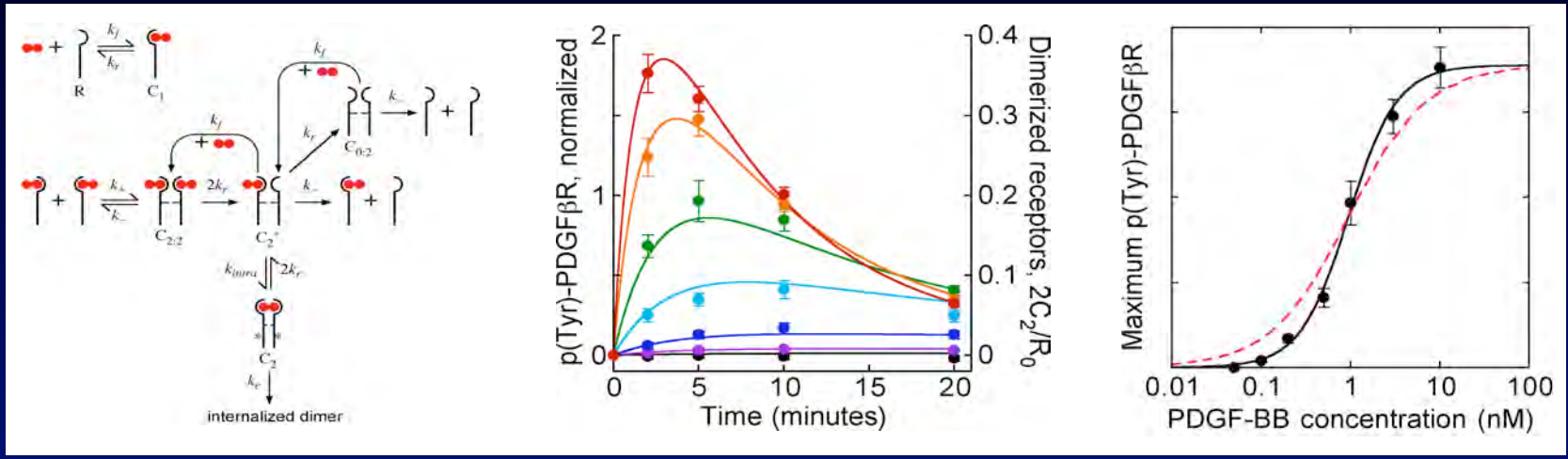
Type IA PI 3-kinases are strongly activated by PDGF receptors…

… and PI 3-kinase pathways are required for PDGF-stimulated responses, including cell motility & chemotaxis.

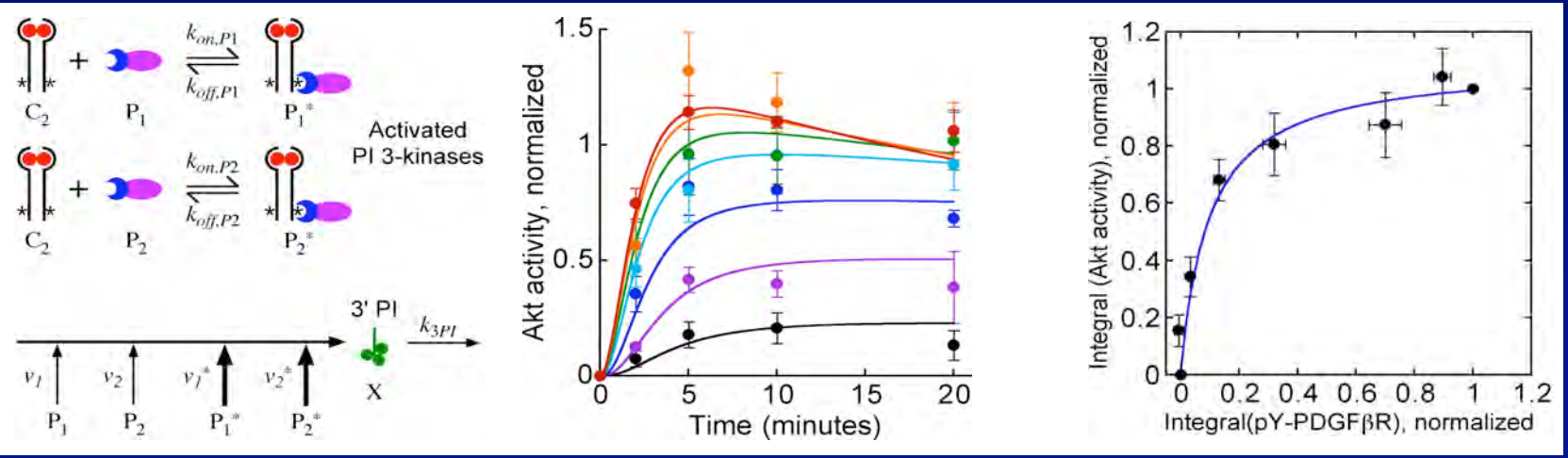


Kinetic analysis of PI 3-kinase activation

The dose response of PDGF receptor activation exhibits positive cooperativity…

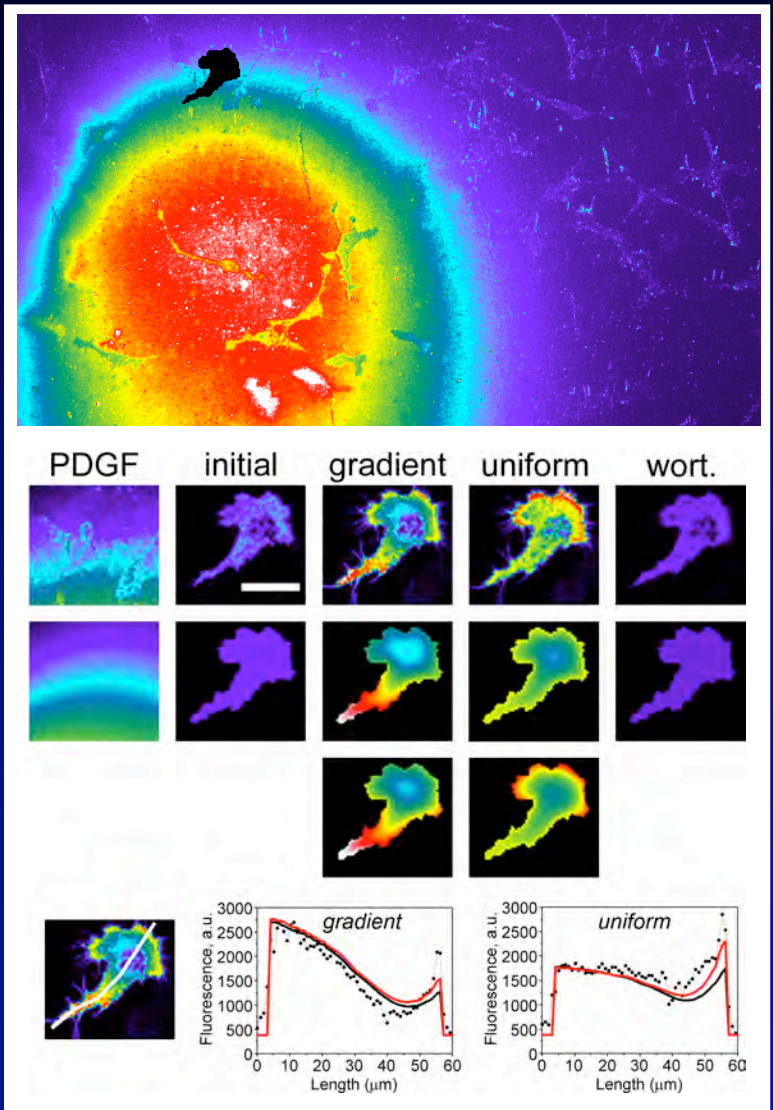


and PI 3-kinase activation is a saturable function of receptor activation.

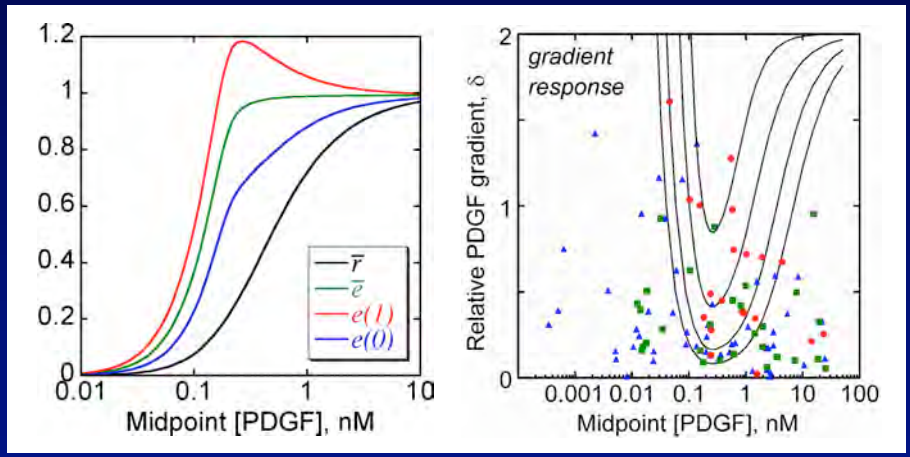


Park et al., *J. Biol. Chem.*, 278: 37064 (2003).

Quantitative elucidation of the PDGF gradient sensing mechanism in fibroblasts (Ian Schneider)



TIRF microscopy and kinetic/spatial modeling were used to characterize the sensitivity of PDGF gradient sensing in fibroblasts.

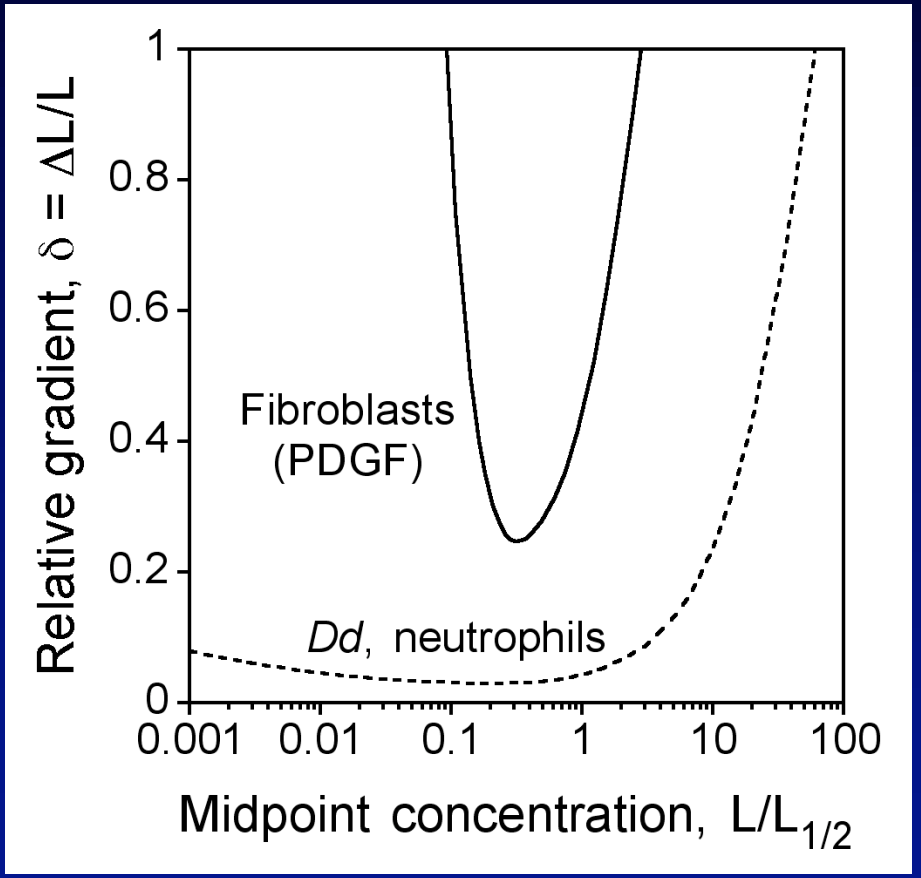


***Biophys. J.*, 86:589 (2004).**
***Biophys. J.*, 86:599 (2004).**
***Biophys. J.*, 89:1420 (2005).**
***J. Cell Biol.*, 171:883 (2005).**
***Chem. Eng. Sci.*, 61:5603 (2006).**
***Cell Cycle*, 5: 1130 (2006).**

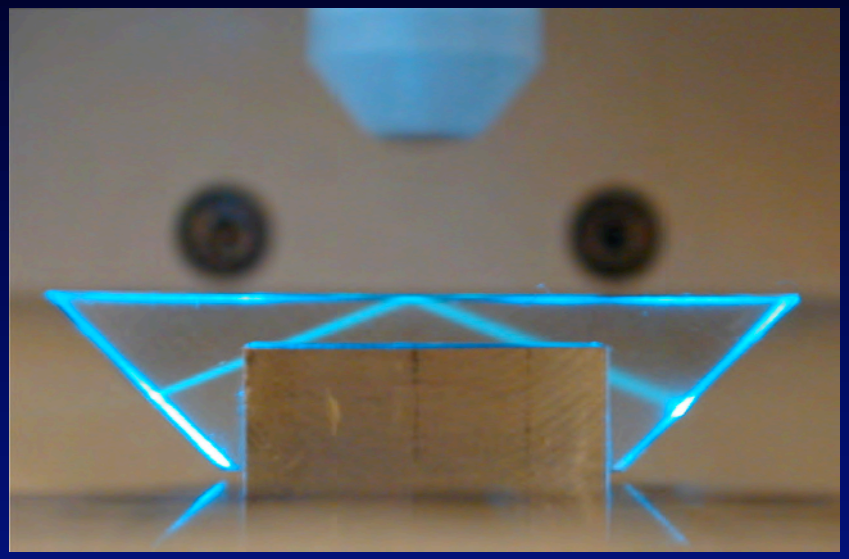
Sensitivity to PDGF gradients: model predictions

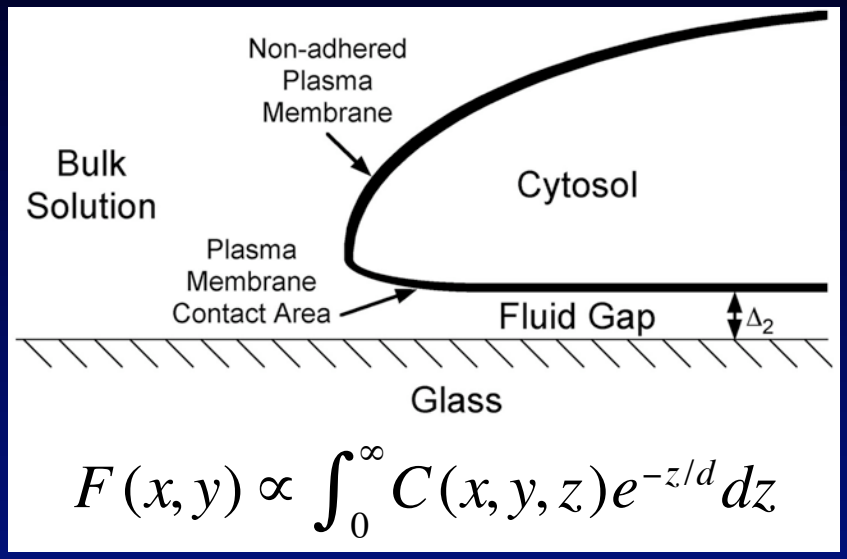
The mechanism of PDGF gradient sensing lacks the features of amplification and adaptation in amoeboid cells.

Robust PDGF gradient sensing requires steeper gradients that span a specific range of midpoint concentrations.

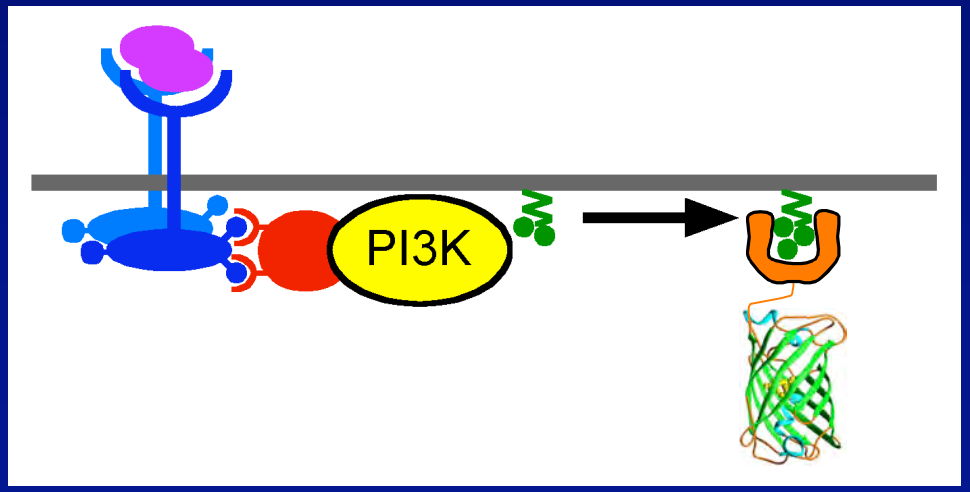


TIRF microscopy

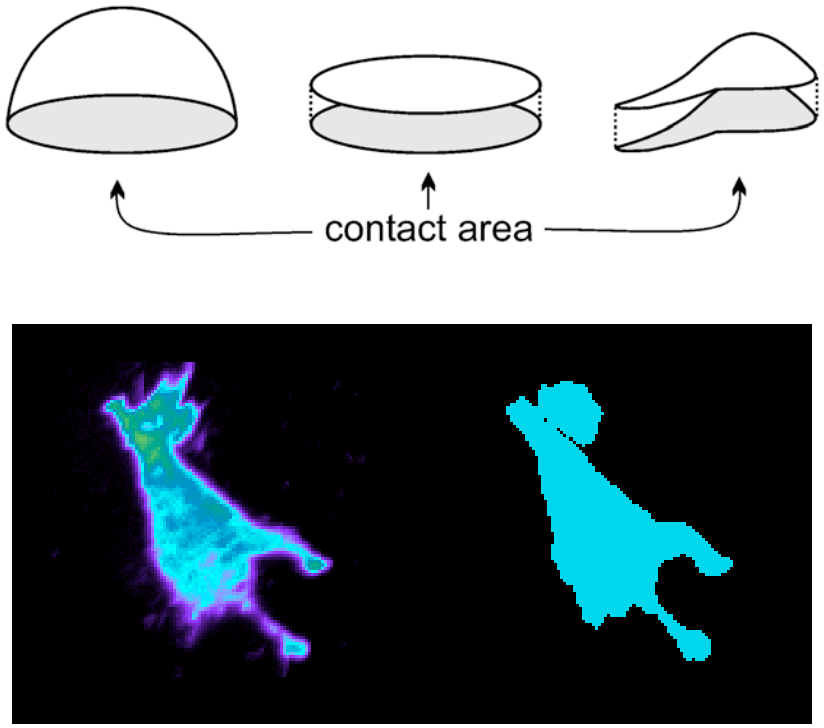


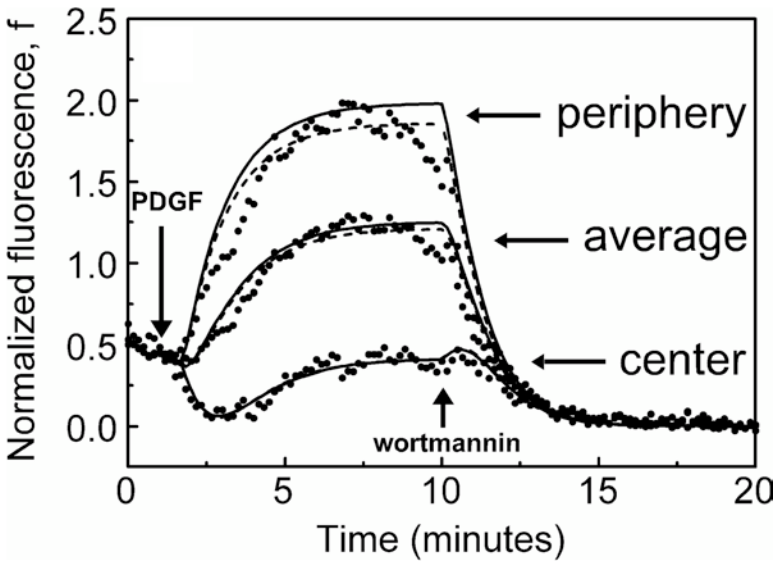


$$F(x,y) \propto \int_0^\infty C(x,y,z)e^{-z/d}dz$$



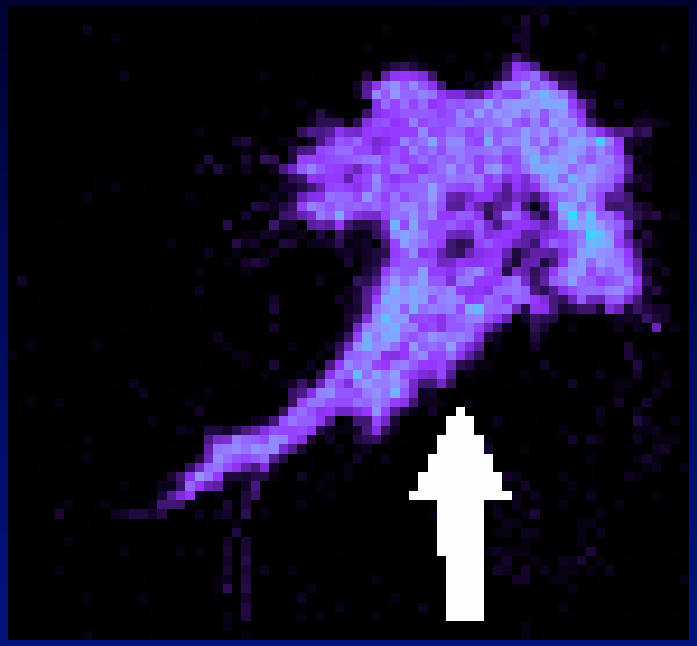
Spatial analysis: uniform stimulation

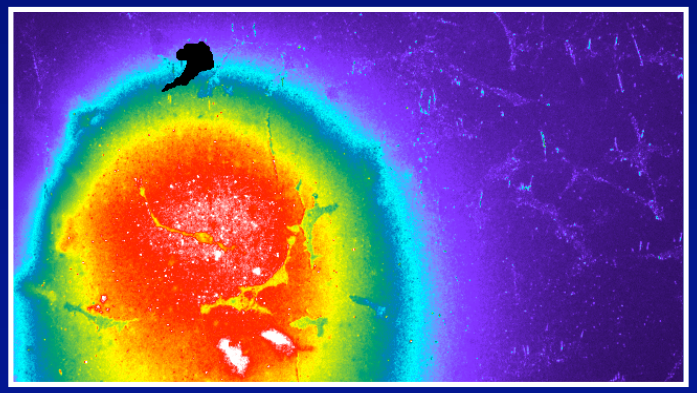




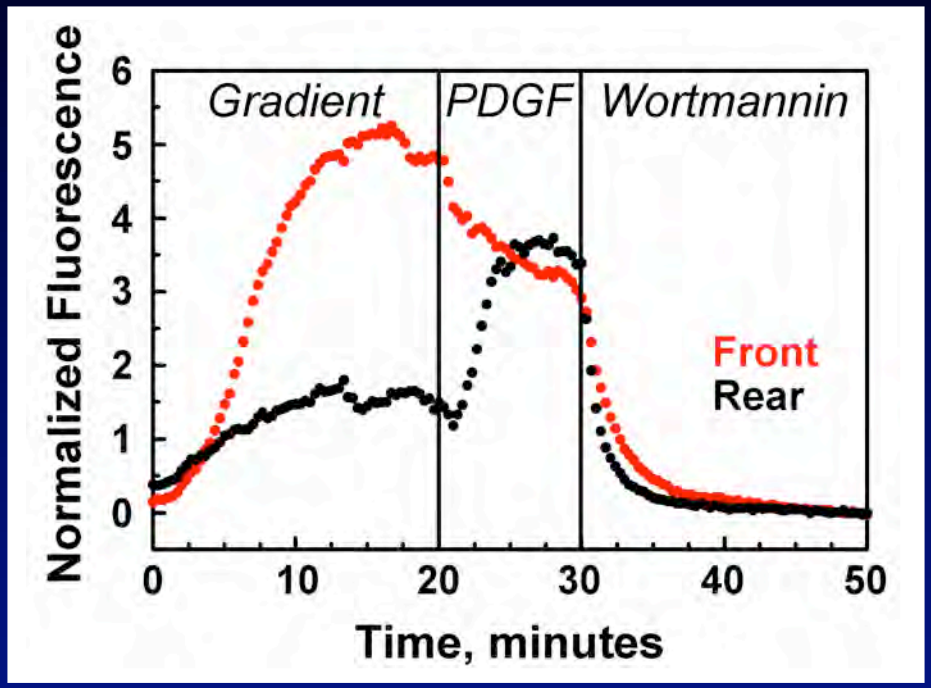
Haugh & Schneider, *Biophys. J.*, 86: 589; Schneider & Haugh, *Biophys. J.*, 86: 599 (2004).
Schneider et al., *Biophys. J.*, 89: 1420 (2005).

Sensitivity to PDGF gradients





Tip: 5nM PDGF

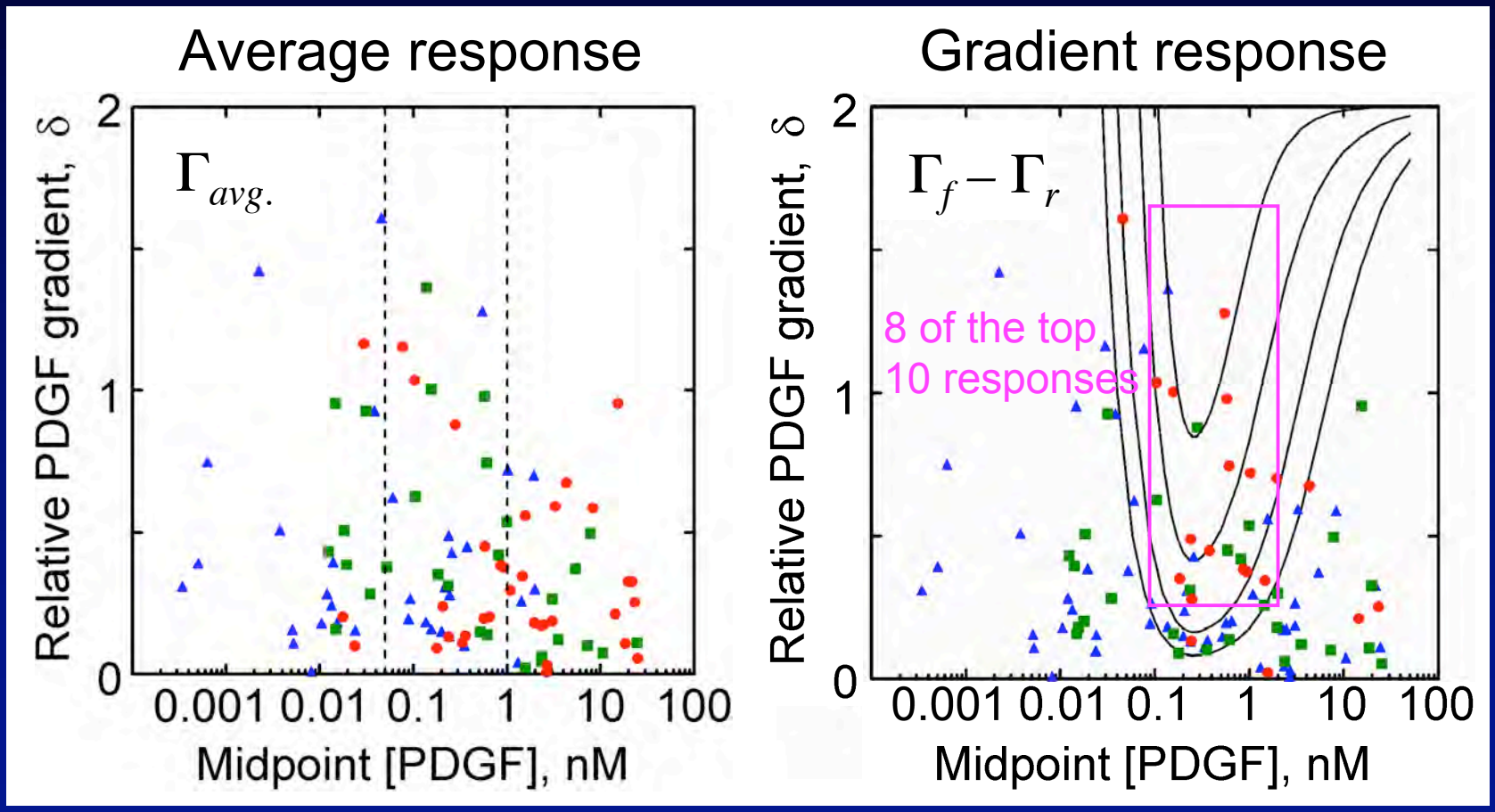


Gradient stimulation is followed by a saturating dose (10 nM), after which wortmannin is added.

Schneider & Haugh. *J. Cell Biol.*, 171: 883 (2005).

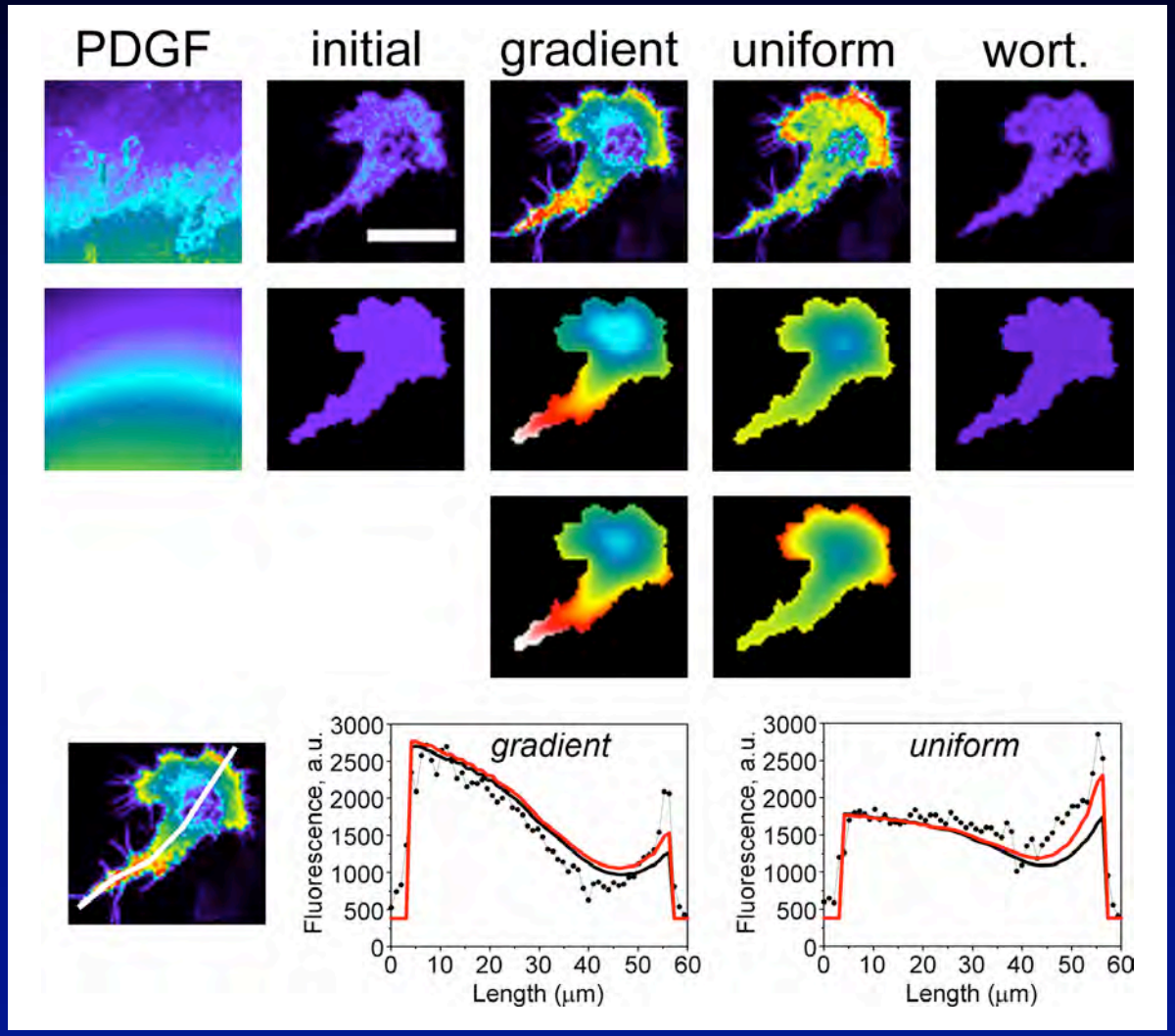
Sensitivity to PDGF gradients

Fractional response: $\Gamma = (f_{gradient} - f_{initial})/(f_{uniform} - f_{initial})$



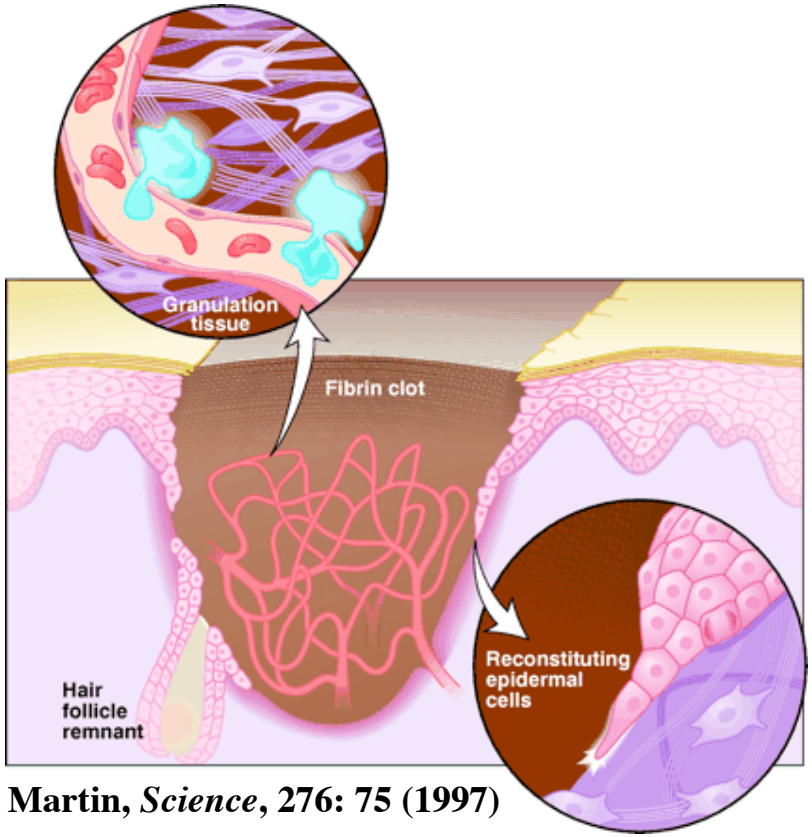
Schneider & Haugh. *J. Cell Biol.*, 171: 883 (2005).

Sensitivity to PDGF gradients

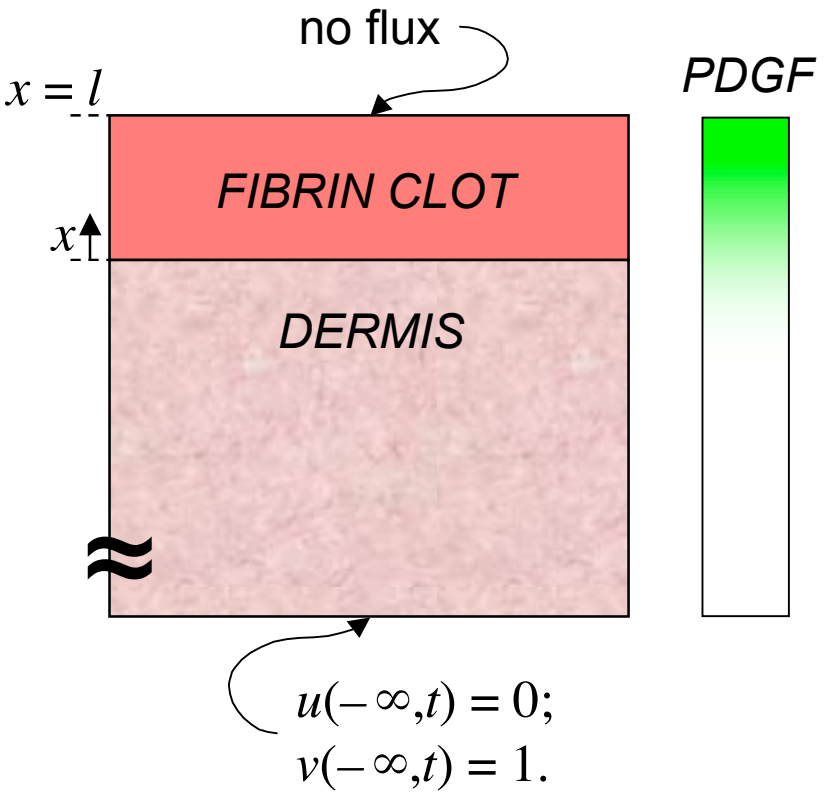


Schneider & Haugh. *J. Cell Biol.*, 171: 883 (2005).
Further analysis: Haugh & Schneider. *Chem. Engr. Sci.*, 61: 5603 (2006).

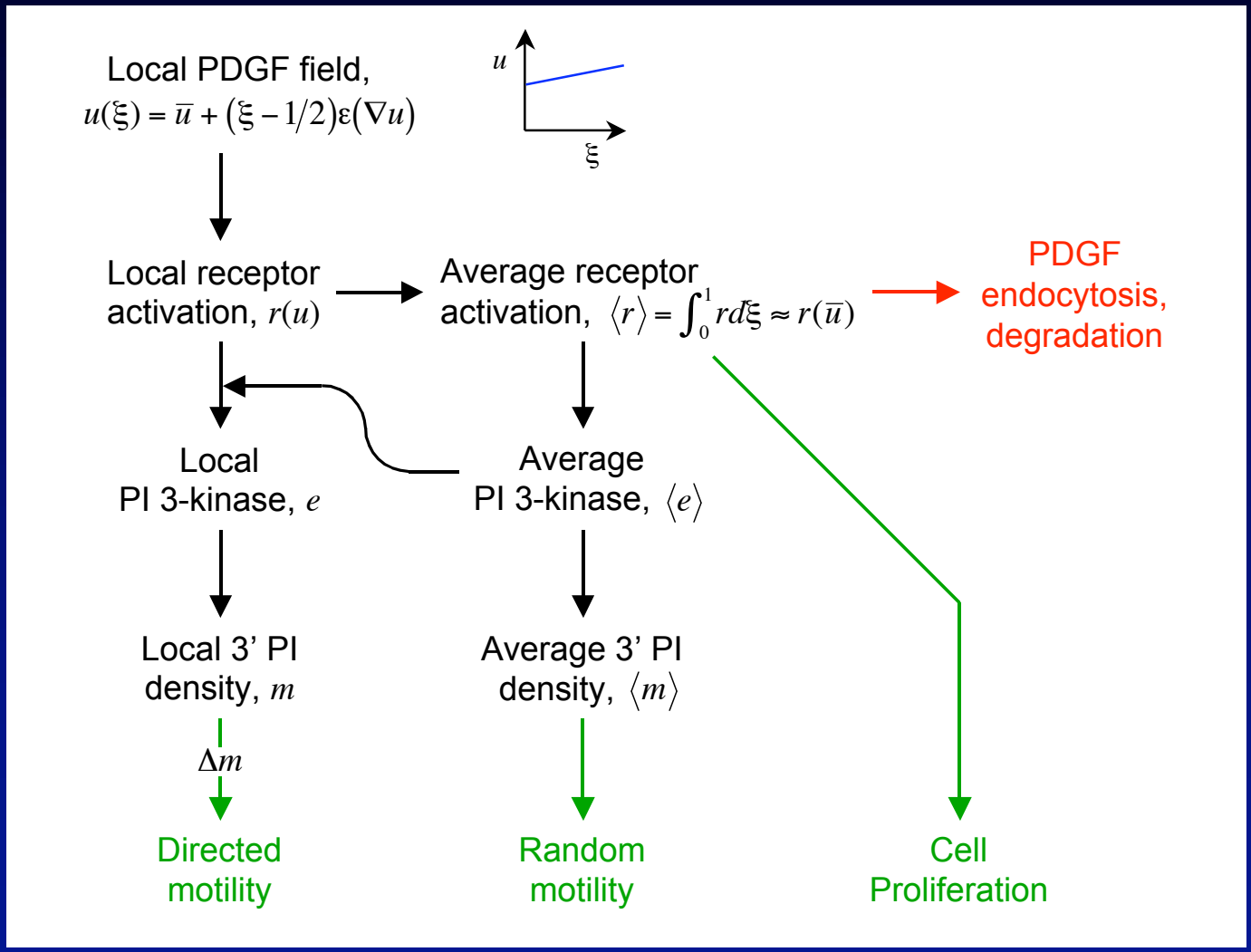
Problem: how is a suitable gradient maintained as the fibroblasts penetrate the wound?



Martin, *Science*, 276: 75 (1997)



Integration of spatial sensing and fibroblast population dynamics



Haugh, *Biophys. J.*, 90: 2297 (2006).

Integration of spatial sensing and fibroblast population dynamics

$$\frac{dR}{dt} = V_s + k_r C_1 + k_-\left(C_2^* + 2C_{0:2}\right) + k_{rec} R_i - \left(k_f[L] + k_t\right)R$$
$$\frac{dC_1}{dt} = k_f[L]R + k_-\left(C_2^* + 2C_{2:2}\right) - \left(k_r + k_t\right)C_1 - 2k_+ C_1^{\,2}$$
$$\frac{dC_{2:2}}{dt} = k_+ C_1^{\,2} + k_f[L]C_2^* - \left(2k_r + k_- + k_t\right)C_{2:2}$$
$$\frac{dC_2^*}{dt} = 2k_r\left(C_{2:2} + C_2\right) + 2k_f[L]C_{0:2} - \left(k_r + k_f[L] + k_- + k_{intra} + k_t\right)C_2^*$$
$$\frac{dC_{0:2}}{dt} = k_r C_2^* - \left(2k_f[L] + k_- + k_t\right)C_{0:2}$$
$$\frac{dC_2}{dt} = k_{intra} C_2^* - \left(2k_r + k_e\right)C_2$$
$$\frac{dR_i}{dt} = k_t\left(R + C_1 + 2C_{2:2} + 2C_2^* + 2C_{0:2}\right) + 2k_e\left(1 - f_D\right)C_2 - \left(k_{rec} + k_{deg}\right)R_i$$

11 rate parameters + receptor balance

"Model compression"

$$r = \frac{C_2}{C_{2,\max}} = \frac{1 + 4\kappa_x\varphi_1\varphi_2 - \left(1 + 8\kappa_x\varphi_1\varphi_2\right)^{1/2}}{\varphi_2^{\,2}\left[1 + 4\kappa_x - \left(1 + 8\kappa_x\right)^{1/2}\right]}$$

$$\varphi_1 = \frac{k_f[L]}{k_r + k_t + k_f[L]}; \quad \varphi_2 = \varphi_1 + \beta\left(1 - \varphi_1\right);$$

$$\kappa_x = \frac{k_e}{k_t}\left(1 + \frac{k_{rec} f_D}{k_{deg}}\right)K_X R_0;$$

$$\beta = \frac{1 + k_{-x}/2k_e}{\left(1 + k_r/k_t\right)\left(1 + k_{rec} f_D/k_{deg}\right)} << 1$$

2 rate parameters

$$r(u) = \frac{u^2}{1 + u + u^2}$$

No parameters

Haugh, *Biophys. J.*, 90: 2297 (2006).

Reconciliation of fibroblast proliferation and fibroblast-mediated PDGF consumption defines the dynamic range of PDGF concentration in the clot

PDGF (u):

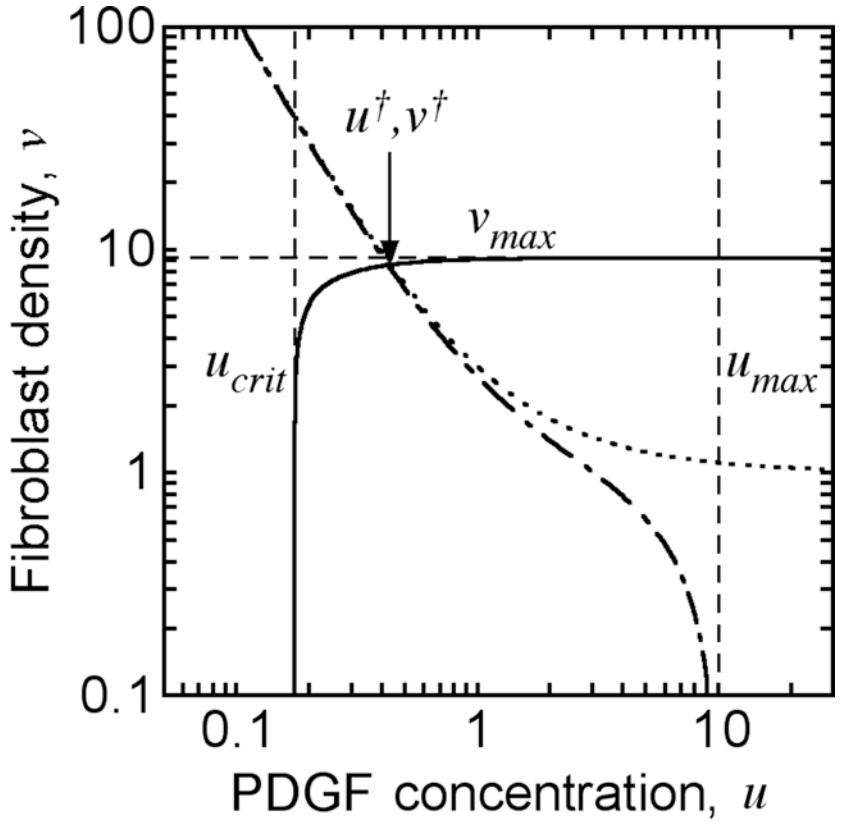
$$\frac{\partial u}{\partial t} = D_u \nabla^2 u + k_s - k_u u - k_v r v$$

Fibroblast density (v):

$$\frac{\partial v}{\partial t} = -\nabla \cdot J_v + R_p;$$

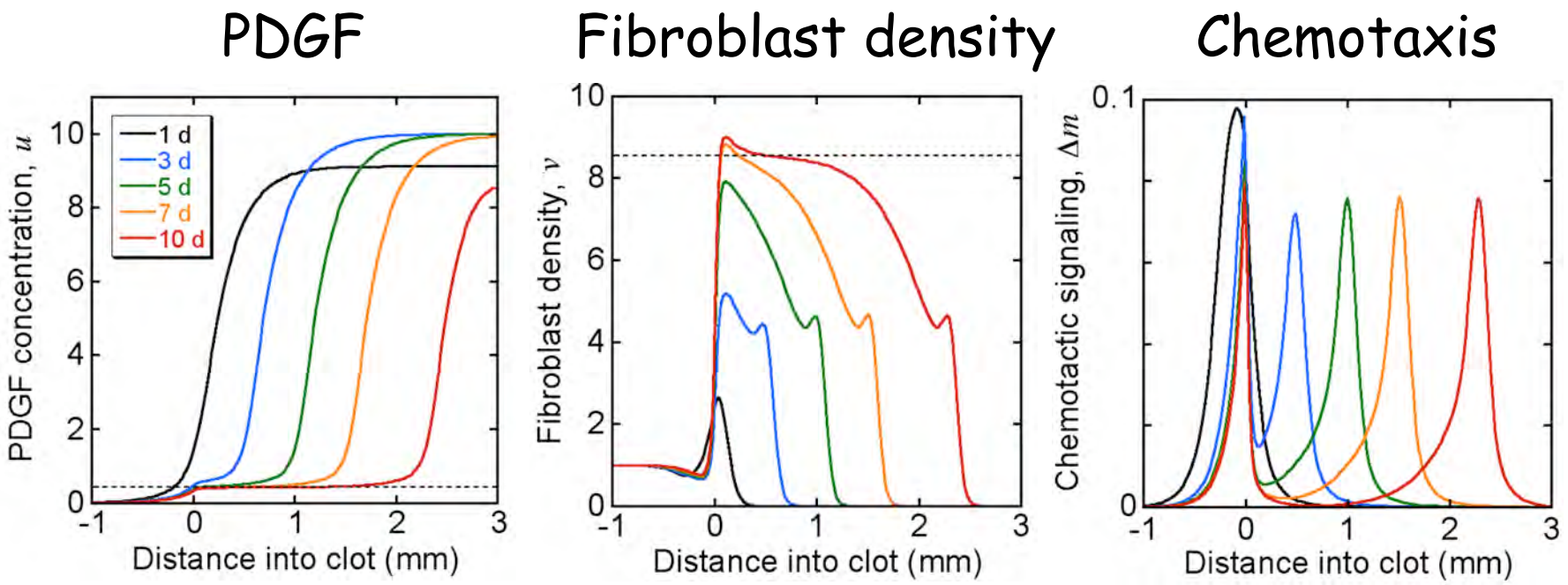
$$R_p = \left(\frac{\mu_m r}{\gamma + r}\right)\left[1 - \left(\frac{v}{v^*}\right)^n\right] v - k_d (v - v_0);$$

$$J_v = -D_v^* \langle m \rangle \nabla v + \left(S_{tax}^* \Delta m - D_v^* \nabla \langle m \rangle\right) v.$$



Haugh, *Biophys. J.*, 90: 2297 (2006).

Fibroblast-mediated PDGF consumption allows for the maintenance of a constant PDGF gradient that propagates in tandem with the invading fibroblast front



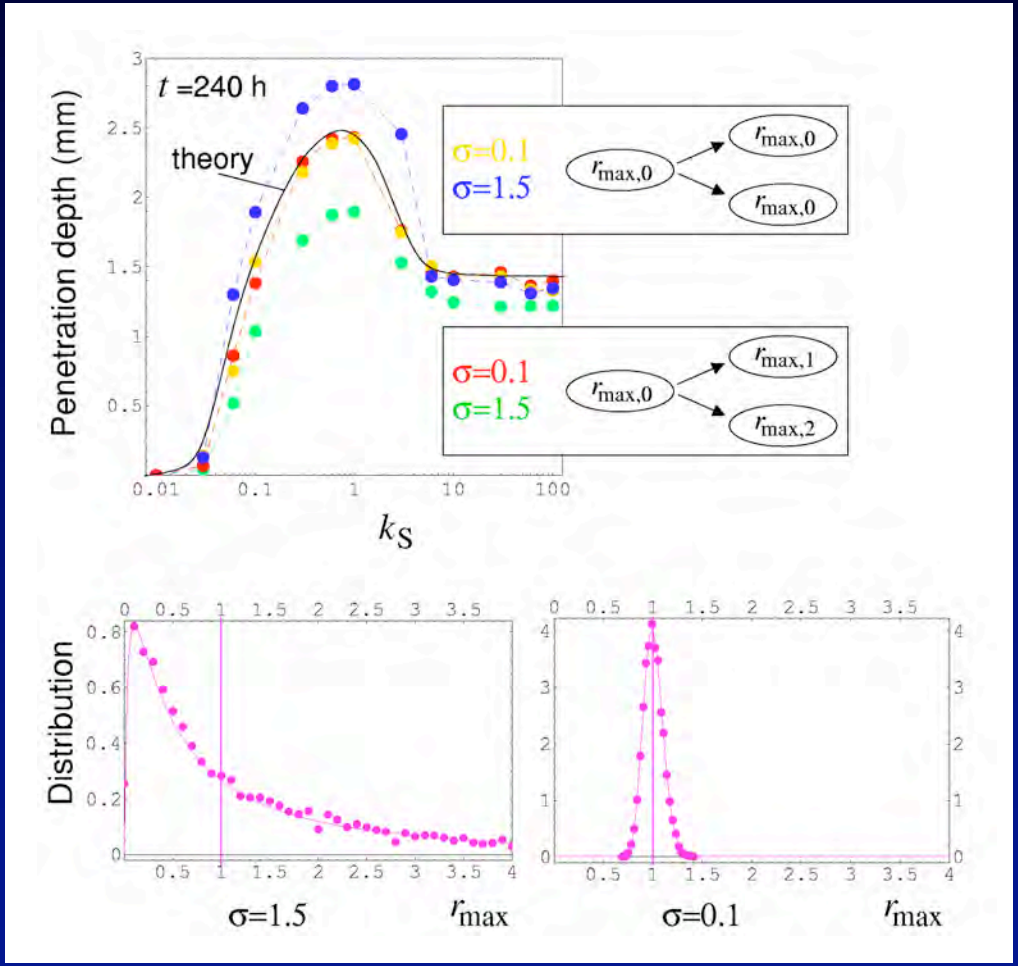
Haugh, *Biophys. J.*, 90: 2297 (2006).

Fibroblast-mediated PDGF consumption allows for maintenance of a constant PDGF gradient that propagates in tandem with the invading fibroblast front

PDGF endocytosis by the leading cohort of fibroblasts is a plausible mechanism for maintenance of PDGF gradients spanning the optimal range.

Haugh, *Biophys. J.*, 90: 2297 (2006).

Does heterogeneity in the fibroblast population affect the invasion rate?



How far the "population" penetrates depends on:

- The distribution of cell properties (e.g., receptor & PI3K expression levels);
- The nature of their inheritance by daughter cells.

When daughter cells inherit the receptor expression of their progenitor, cells with different expression levels are separated, resulting in a higher rate of invasion.

Monine & Haugh, in preparation.

Summary

• At the molecular level, cell surface receptors mediate the early stages of signal transduction by providing a scaffold for assembly of multi-protein complexes and for modification of cell membrane-associated signaling molecules.

• At the cellular level, receptor -mediated signaling may be localized so as to direct migration along chemoattractant gradients. The PDGF gradient sensing mechanism lacks the feedback loops that yield signal amplification and adaptation in amoeboid cells. Robust PDGF gradient sensing requires steeper gradients with midpoint concentrations that yield near maximal PI 3-kinase activation without saturating PDGF receptor binding.

• At the tissue level, modeling of the wound invasion process shows that a constant PDGF gradient, spanning the optimal concentration range, can be maintained through receptor-mediated PDGF clearance at the leading fibroblast front.

Thanks

Haugh Lab: current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Matej Krajcovic


Adam Melvin


Dr. Rajiv Vaidya


Chun-Chao Wang


Michael Weiger

Alumni


Misha Monine


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